

Claims:

1. A process for the preparation of zeaxanthin wherein said process comprises culturing a recombinant cell containing farnesyl pyrophosphate and isopentyl pyrophosphate under culture conditions sufficient for the expression of enzymes  
5 which catalyze the conversion of the farnesyl pyrophosphate and isopentyl pyrophosphate to zeaxanthin, said recombinant cell being a host cell transformed by an expression vector comprising a regulatory sequence and a polynucleotide containing the following DNA sequences which encode said enzymes:

a) a DNA sequence which encodes the GGPP synthase of *Flavobacterium* sp. R1534 (crtE) or a DNA sequence which is substantially homologous,  
10

b) a DNA sequence which encodes the prephytoene synthase of *Flavobacterium* sp. R1534 (crtB) or a DNA sequence which is substantially homologous,

c) a DNA sequence which encodes the phytoene desaturase of  
15 *Flavobacterium* sp. R1534 (crtI) or a DNA sequence which is substantially homologous,

d) a DNA sequence which encodes the lycopene cyclase of *Flavobacterium* sp. R1534 (crtY) or a DNA sequence which is substantially homologous,

e) a DNA sequence which encodes the  $\beta$ -carotene hydroxylase of  
20 microorganism E-396 (crtZE396) or a DNA sequence which is substantially homologous;

and isolating the zeaxanthin from such cells or the culture medium.

2. The process of claim 1 wherein said DNA sequences are:

a) a DNA sequence which encodes the GGPP synthase of *Flavobacterium* sp. R1534 (crtE),  
25

b) a DNA sequence which encodes the prephytoene synthase of *Flavobacterium* sp. R1534 (crtB),

c) a DNA sequence which encodes the phytoene desaturase of *Flavobacterium* sp. R1534 (crtI),

d) a DNA sequence which encodes the lycopene cyclase of *Flavobacterium* sp. R1534 (crtY), and

e) a DNA sequence which encodes the  $\beta$ -carotene hydroxylase of microorganism E-396 (crtZE396).

5        3. The process of claim 2 wherein:

a) the GGPP synthase has the amino acid sequence of Figure 8,

b) the prephytoene synthase has the amino acid sequence of Figure 9,

c) the phytoene desaturase has the amino acid sequence of Figure 10,

d) the lycopene cyclase has the amino acid sequence of Figure 11, and

10       e) the  $\beta$ -carotene hydroxylase has the amino acid sequence of Figure 34.

4. The process of claim 3 wherein:

a) the DNA sequence encoding the GGPP synthase comprises bases 2521-3408 of Figure 7,

15       b) the DNA sequence encoding the prephytoene synthase comprises bases 4316-3405 of Figure 7,

c) the DNA sequence encoding the phytoene desaturase comprises bases 4313-5797 of Figure 7,

d) the DNA sequence encoding the lycopene cyclase comprises bases 5794-6942 of Figure 7, and

20       e) the DNA sequence encoding the  $\beta$ -carotene hydroxylase comprises the sequence of Figure 33.

25       5. A process for the preparation of canthaxanthin wherein said process comprises culturing a recombinant cell containing farnesyl pyrophosphate and isopentyl pyrophosphate under culture conditions sufficient for the expression of enzymes which catalyze the conversion of the farnesyl pyrophosphate and isopentyl pyrophosphate to canthaxanthin, said recombinant cell being a host cell transformed by an expression vector comprising a regulatory sequence and a

polynucleotide containing the following DNA sequences which encode said enzymes:

- a) a DNA sequence which encodes the GGPP synthase of *Flavobacterium* sp. R1534 (crtE) or a DNA sequence which is substantially homologous,
  - 5      b) a DNA sequence which encodes the prephytoene synthase of *Flavobacterium* sp. R1534 (crtB) or a DNA sequence which is substantially homologous,
  - c) a DNA sequence which encodes the phytoene desaturase of *Flavobacterium* sp. R1534 (crtI) or a DNA sequence which is substantially  
10 homologous,
  - d) a DNA sequence which encodes the lycopene cyclase of *Flavobacterium* sp. R1534 (crtY) or a DNA sequence which is substantially homologous, and
  - e) a DNA sequence which encodes the  $\beta$ -carotene  $\beta$ 4-oxygenase of microorganism E-396 (crtW<sub>E396</sub>) or a DNA sequence which is substantially  
15 homologous;
- and isolating the canthaxanthin from such cells or the culture medium.

6. The process of claim 5 wherein said DNA sequences are:

- a) a DNA sequence which encodes the GGPP synthase of *Flavobacterium* sp. R1534 (crtE),
- 20      b) a DNA sequence which encodes the prephytoene synthase of *Flavobacterium* sp. R1534 (crtB),
- c) a DNA sequence which encodes the phytoene desaturase of *Flavobacterium* sp. R1534 (crtI),
- d) a DNA sequence which encodes the lycopene cyclase of *Flavobacterium* sp. R1534 (crtY), and  
25
- e) a DNA sequence which encodes the  $\beta$ -carotene  $\beta$ 4-oxygenase of microorganism E-396 (crtW<sub>E396</sub>).

7. The process of claim 6 wherein:

- a) the GGPP synthase has the amino acid sequence of Figure 8,
- b) the prephytoene synthase has the amino acid sequence of Figure 9,
- c) the phytoene desaturase has the amino acid sequence of Figure 10,
- d) the lycopene cyclase has the amino acid sequence of Figure 11, and
- 5 e) the  $\beta$ -carotene  $\beta$ 4-oxygenase has the amino acid sequence of Figure 32.

8. The process of claim 7 wherein:

- a) the DNA sequence encoding the GGPP synthase comprises bases 2521-3408 of Figure 7,
- 10 b) the DNA sequence encoding the prephytoene synthase comprises bases 4316-3405 of Figure 7,
- c) the DNA sequence encoding the phytoene desaturase comprises bases 4313-5797 of Figure 7,
- d) the DNA sequence encoding the lycopene cyclase comprises bases 5794-6942 of Figure 7, and
- 15 e) the DNA sequence encoding the  $\beta$ -carotene  $\beta$ 4-oxygenase comprises the sequence of Figure 31.

9. A process for the preparation of astaxanthin and adonixanthin wherein said process comprises culturing a recombinant cell containing farnesyl pyrophosphate and isopentyl pyrophosphate under culture conditions sufficient  
20 for the expression of enzymes which catalyze the conversion of the farnesyl pyrophosphate and isopentyl pyrophosphate to astaxanthin and adonixanthin, said recombinant cell being a host cell transformed by an expression vector comprising a regulatory sequence and a polynucleotide containing the following DNA sequences which encode said enzymes:

- 25 a) a DNA sequence which encodes the GGPP synthase of *Flavobacterium* sp. R1534 (crtE) or a DNA sequence which is substantially homologous,

- b) a DNA sequence which encodes the prephytoene synthase of *Flavobacterium* sp. R1534 (crtB) or a DNA sequence which is substantially

homologous,

c) a DNA sequence which encodes the phytoene desaturase of *Flavobacterium* sp. R1534 (crtI) or a DNA sequence which is substantially homologous,

5 d) a DNA sequence which encodes the lycopene cyclase of *Flavobacterium* sp. R1534 (crtY) or a DNA sequence which is substantially homologous,

e) a DNA sequence which encodes the  $\beta$ -carotene  $\beta$ 4-oxygenase of *Alcaligenes* PC-1 (crtW) or a DNA sequence which is substantially homologous, and

10 f) a DNA sequence which encodes the  $\beta$ -carotene hydroxylase of microorganism E-396 (crtZE396) or a DNA sequence which is substantially homologous;

and isolating the astaxanthin and adonixanthin from such cells or the culture medium.

10. The process of claim 9 wherein said DNA sequences are:

15 a) a DNA sequence which encodes the GGPP synthase of *Flavobacterium* sp. R1534 (crtE),

b) a DNA sequence which encodes the prephytoene synthase of *Flavobacterium* sp. R1534 (crtB),

20 c) a DNA sequence which encodes the phytoene desaturase of *Flavobacterium* sp. R1534 (crtI),

d) a DNA sequence which encodes the lycopene cyclase of *Flavobacterium* sp. R1534 (crtY),

e) a DNA sequence which encodes the  $\beta$ -carotene  $\beta$ 4-oxygenase of *Alcaligenes* PC-1 (crtW), and

25 f) a DNA sequence which encodes the  $\beta$ -carotene hydroxylase of microorganism E-396 (crtZE396).

11. The process of claim 10 wherein:

- a) the GGPP synthase has the amino acid sequence of Figure 8,
- b) the prephytoene synthase has the amino acid sequence of Figure 9,
- c) the phytoene desaturase has the amino acid sequence of Figure 10,
- d) the lycopene cyclase has the amino acid sequence of Figure 11,
- 5 e) the  $\beta$ -carotene  $\beta$ 4-oxygenase has the amino acid sequence of Figure 25, and
- f) the  $\beta$ -carotene hydroxylase has the amino acid sequence of Figure 34.

12. The process of claim 11 wherein:

- a) the DNA sequence encoding the GGPP synthase comprises bases 2521-3408 of Figure 7,
- 10 b) the DNA sequence encoding the prephytoene synthase comprises bases 4316-3405 of Figure 7,
- c) the DNA sequence encoding the phytoene desaturase comprises bases 4313-5797 of Figure 7,
- d) the DNA sequence encoding the lycopene cyclase comprises bases 5794-6942
- 15 of Figure 7,
- e) the DNA sequence encoding the  $\beta$ -carotene  $\beta$ 4-oxygenase comprises the sequence of Figure 25, and
- f) the DNA sequence encoding the  $\beta$ -carotene hydroxylase comprises the sequence of Figure 33.

- 20 13. A process for the preparation of astaxanthin and adonixanthin wherein said process comprises culturing a recombinant cell containing farnesyl pyrophosphate and isopentyl pyrophosphate under culture conditions sufficient for the expression enzymes which catalyze the conversion of the farnesyl pyrophosphate and isopentyl pyrophosphate to astaxanthin and adonixanthin,
- 25 said recombinant cell being a host cell transformed by an expression vector comprising a regulatory sequence and a polynucleotide containing the following DNA sequences which encode said enzymes:

a) a DNA sequence which encodes the GGPP synthase of *Flavobacterium* sp. R1534 (crtE) or a DNA sequence which is substantially homologous,

5 b) a DNA sequence which encodes the prephytoene synthase of *Flavobacterium* sp. R1534 (crtB) or a DNA sequence which is substantially homologous,

c) a DNA sequence which encodes the phytoene desaturase of *Flavobacterium* sp. R1534 (crtI) or a DNA sequence which is substantially homologous,

10 d) a DNA sequence which encodes the lycopene cyclase of *Flavobacterium* sp. R1534 (crtY) or a DNA sequence which is substantially homologous,

e) a DNA sequence which encodes the  $\beta$ -carotene b4-oxygenase of microorganism E-396 (crtWE396) or a DNA sequence which is substantially homologous, and

15 f) a DNA sequence which encodes the  $\beta$ -carotene hydroxylase of microorganism E-396 (crtZE396) or a DNA sequence which is substantially homologous;

and isolating the astaxanthin and adonixanthin from such cells or the culture medium.

14. The process of claim 13 wherein said DNA sequences are:

20 a) a DNA sequence which encodes the GGPP synthase of *Flavobacterium* sp. R1534 (crtE),

b) a DNA sequence which encodes the prephytoene synthase of *Flavobacterium* sp. R1534 (crtB),

25 c) a DNA sequence which encodes the phytoene desaturase of *Flavobacterium* sp. R1534 (crtI),

d) a DNA sequence which encodes the lycopene cyclase of *Flavobacterium* sp. R1534 (crtY),

e) a DNA sequence which encodes the  $\beta$ -carotene  $\beta$ 4-oxygenase of microorganism E-396 (crtWE396), and

f) a DNA sequence which encodes the  $\beta$ -carotene hydroxylase of microorganism E-396 (crtZE396).

5        15. The process of claim 14 wherein:

a) the GGPP synthase has the amino acid sequence of Figure 8,

b) the prephytoene synthase has the amino acid sequence of Figure 9,

c) the phytoene desaturase has the amino acid sequence of Figure 10,

d) the lycopene cyclase has the amino acid sequence of Figure 11,

10        e) the  $\beta$ -carotene  $\beta$ 4-oxygenase has the amino acid sequence of Figure 32, and

f) the  $\beta$ -carotene hydroxylase has the amino acid sequence of Figure 34.

16. The process of claim 15 wherein:

a) the DNA sequence encoding the GGPP synthase comprises bases 2521-3408 of Figure 7,

15        b) the DNA sequence encoding the prephytoene synthase comprises bases 4316-3405 of Figure 7,

c) the DNA sequence encoding the phytoene desaturase comprises bases 4313-5797 of Figure 7,

20        d) the DNA sequence encoding the lycopene cyclase comprises bases 5794-6942 of Figure 7,

e) the DNA sequence encoding the  $\beta$ -carotene  $\beta$ 4-oxygenase comprises the sequence of Figure 31, and

f) the DNA sequence encoding the  $\beta$ -carotene hydroxylase comprises the sequence of Figure 33.

25        17. A process for the preparation of adonixanthin wherein said process comprises culturing a recombinant cell containing farnesyl pyrophosphate and



isopentyl pyrophosphate under culture conditions sufficient for the expression of enzymes which catalyze the conversion of the farnesyl pyrophosphate and isopentyl pyrophosphate to adonixanthin, said recombinant cell being a host cell transformed by an expression vector comprising a regulatory sequence and a  
5 polynucleotide containing the following DNA sequences which encode said enzymes:

- a) a DNA sequence which encodes the GGPP synthase of microorganism E-396 (crtE<sub>E396</sub>) or a DNA sequence which is substantially homologous,
- b) a DNA sequence which encodes the prephytoene synthase of  
10 microorganism E-396 (crtB<sub>E396</sub>) or a DNA sequence which is substantially homologous,
- c) a DNA sequence which encodes the phytoene desaturase of microorganism E-396 (crtI<sub>E396</sub>) or a DNA sequence which is substantially homologous,
- d) a DNA sequence which encodes the lycopene cyclase of microorganism E-  
15 396 (crtY<sub>E396</sub>) or a DNA sequence which is substantially homologous,
- e) a DNA sequence which encodes the b-carotene b4-oxygenase of microorganism E-396 (crtW<sub>E396</sub>) or a DNA sequence which is substantially homologous, and
- f) a DNA sequence which encodes the  $\beta$ -carotene hydroxylase of  
20 microorganism E-396 (crtZ<sub>E396</sub>) or a DNA sequence which is substantially homologous,

said host cell being substantially free of other polynucleotides of microorganism E-396;

and isolating the adonixanthin from such cells or the culture medium.

25 18. The process of claim 17 wherein said DNA sequences are:

- a) a DNA sequence which encodes the GGPP synthase of microorganism E-396 (crtE<sub>E396</sub>),
- b) a DNA sequence which encodes the prephytoene synthase of microorganism E-396 (crtB<sub>E396</sub>),

c) a DNA sequence which encodes the phytoene desaturase of microorganism E-396 (crtI<sub>E396</sub>),

d) a DNA sequence which encodes the lycopene cyclase of microorganism E-396 (crtY<sub>E396</sub>),

5 e) a DNA sequence which encodes the  $\beta$ -carotene  $\beta$ 4-oxygenase of microorganism E-396 (crtW<sub>E396</sub>), and

f) a DNA sequence which encodes the  $\beta$ -carotene hydroxylase of microorganism E-396 (crtZ<sub>E396</sub>).

10 19. The process of claim 18 wherein the polynucleotide is plasmid pE396CARcrtW-E.

\*\*\*